Fall in intracellular Po2 at the onset of contractions in Xenopus single skeletal muscle fibers

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**Hogan, Michael C.** Fall in intracellular Po2 at the onset of contractions in Xenopus single skeletal muscle fibers. *J Appl Physiol* 90: 1871–1876, 2001.— It remains uncertain whether the delayed onset of mitochondrial respiration on initiation of muscle contractions is related to O2 availability. The purpose of this research was to measure the kinetics of the fall in intracellular Po2 at the onset of a contractile work period in rested and previously worked single skeletal muscle fibers. Intact single skeletal muscle fibers (n = 11) from Xenopus laevis were dissected from the lumbrical muscle, injected with an O2-sensitive probe, mounted in a glass chamber, and perfused with Ringer solution (Po2 = 32 ± 4 Torr and pH = 7.0) at 20°C. Intracellular Po2 was measured in each fiber during a protocol consisting sequentially of 1-min rest; 3 min of tetanic contractions (1 contraction/2 s); 5-min rest; and, finally, a second 3-min contractile period identical to the first. Maximal force development and the fall in force (to 83% of maximal force development) at the onset of contractions was significantly greater (P < 0.01) in the first contractile period (13 ± 3 s) compared with the second (5 ± 2 s), as was the time to reach 50% of the contractile steady-state intracellular Po2 (28 ± 5 vs. 18 ± 4 s, respectively). In Xenopus single skeletal muscle fibers, 1) the lengthy response time for the fall in intracellular Po2 at the onset of contractions suggests that intracellular factors other than O2 availability determine the on-kinetics of oxidative phosphorylation and 2) a prior contractile period results in more rapid on-kinetics.

There has been considerable debate concerning the factors that determine the time course of the increase in O2 uptake (Vo2 on-kinetics) with the initiation of exercise or muscle contractions (for review see Ref. 28). It remains uncertain whether the delivery of O2 to the mitochondria is limiting at the onset of steady-state contractions, perhaps related to adaptation of the cardiovascular and microcirculatory systems, or whether mitochondrial O2 availability is adequate, and therefore changes in other regulators of oxidative phosphorylation determine the mitochondrial response time. We recently demonstrated that convective O2 delivery to muscle and peripheral O2 diffusion do not represent limiting factors for the muscle Vo2 on-kinetics (10, 11) during transitions from rest to contractions corresponding to ~60% of the muscle peak Vo2 in isolated muscle. These findings are in agreement with the hypothesis that the limiting factors for Vo2 on-kinetics at these submaximal work intensities are not related to insufficient O2 available for mitochondrial respiration but likely reside within alternate regulatory pathways controlling skeletal muscle oxidative metabolism (4, 5, 19, 32). However, evidence obtained in exercising humans (8, 13) and in contracting isolated muscle (12) has demonstrated that O2 delivery to muscle may be one of the limiting factors for VO2 on-kinetics at higher exercise intensities. In addition, other studies (9, 16) suggest that muscle that has been previously exercised may exhibit faster Vo2 on-kinetics, but it remains unclear whether this is due to faster delivery of O2 to the mitochondria or faster activation of key enzymatic processes within the contracting cells that cause a more rapid activation of oxidative phosphorylation.

Therefore, the purpose of the present study was to use contracting isolated single skeletal muscle fibers to test the hypotheses that 1) O2 supply to the mitochondria of these muscle fibers is not limiting at the onset of steady-state contractions, thereby suggesting that other intracellular regulators of oxidative phosphorylation determine Vo2 on-kinetics, and 2) a prior period of muscle stimulation results in a more rapid activation of oxidative phosphorylation, which also is a result of intracellular processes not related to O2 availability. These hypotheses were tested by using a novel technique (14) of measuring intracellular Po2 in these single fibers so that intracellular oxygenation could be monitored at the onset of contractions.

**METHODS**

**Experimental preparation.** Adult female Xenopus laevis were doubly pithed and decapitated. Lumbrical muscles II-IV were removed, and single living muscle fibers (n = 11) were microdissected from the muscle. Dissections and experiments were performed in Ringer solution (in mM: 112 NaCl, 1.87 KCl, 0.82 CaCl2, 2.38 NaHCO3, 0.07 NaH2PO4, and 0.1 EGTA) at 20°C and 7.0 pH. After dissection, platinum clips were attached to the tendons and the fibers were mounted in
a glass chamber. A solution of 10 mM fura 2 (Molecular Probes, Eugene, OR; for visual monitoring of the injection using excitation light at 390 nm) and 0.5 mM palladium-meso-tetra (4-carboxyphenyl) porphine bound to bovine serum albumin was injected into each single fiber by micropipette pressure injection. One tendon end was then attached to a force transducer system (model 400A, Aurora Scientific, Aurora, Ontario) for measurement of force development. The chamber was then placed on the stage of an inverted microscope configured for epifluorescence. The preparation was observed with a Nikon Fluor objective (×40, 0.70 numerical aperture) used dry. The fiber length was adjusted to achieve maximal force development (P₀) for a single-twitch contraction.

Experimental protocol. Each fiber was continually perfused with Ringer solution that had been equilibrated with a gas mixture to produce a P₀₂ of ~30 Torr and a P₂CO₂ of ~40 Torr. The value of P₀₂ was chosen as representative of a mean capillary P₀₂ that would surround working muscle fibers in vivo. Constant perfusion was maintained during each contractile period to maintain the experimental P₀₂ and to reduce the possible occurrence of unstirred layers surrounding the cell. After a 1-min rest period, tetanic contractions were induced by end-to-end stimulation (50 impulses/s of 1-ms duration at 9 V, with a train duration of 250 ms) using a Grass S48 stimulator (Quincy, MA). Each fiber was stimulated to contract at a frequency of one contraction every 2 s for a period of 3 min. After this first contraction period, each fiber was allowed to rest for 5 min. Immediately after the 5-min rest period, the fiber was again stimulated to contract at a frequency of one contraction every 2 s for 3 min.

A Biopac Systems MP100WSW (Santa Barbara, CA) analog-to-digital converter was used to transform the analog force signal, and the digital data were collected and analyzed with AcqKnowledgeIII 3.5 software (Biopac Systems). Force was recorded in units of force per cross-sectional area (kPa). Individual peak force in a 3-min contraction period was compared with P₀ within that work run and reported as a relative percent. In some experiments, the P₀₂ of the Ringer solution in the chamber was monitored with a Clark-style electrode (model 733, Diamond General, Ann Arbor, MI) placed adjacent to the working fiber.

Intracellular P₀₂ measurement. A new technique (14), using a porphyrin O₂ probe, was used to measure P₀₂ within these single skeletal muscle fibers. The phosphorescence quenching of the palladium-porphyrin O₂ probe within each cell was measured through a system consisting of a flash lamp (Oxygen Enterprises, Philadelphia, PA), a 425-nm band-pass excitation filter, a 630-nm cut-on emission filter, and a photomultiplier tube for collection of the phosphorescence signal. To calculate phosphorescence lifetimes from the intracellular O₂ probe, the phosphorescent decay curves from a series of 10 flashes (15 Hz) were averaged, and a monoexponential function was fit to the subsequent best-fit decay curve (analysis software from Medical Systems, Greenvile, NY). Phosphorescent-decay curves were recorded every 7 s from each cell throughout the experimental period. Previously determined values for the measured phosphorescence lifetime decay in a zero-O₂ environment and the phosphorescence quenching constant for the intracellular O₂ probe were used to calculate intracellular P₀₂ (14).

The individual intracellular P₀₂ data points for each contractile period were fitted by a monoexponential function for subsequent comparison of the intracellular P₀₂ on-kinetics between the two contractile periods. The curve fitting was begun at the time point at which intracellular P₀₂ began to fall after initiation of contractions. In all cells, there was a time delay (TD) after the onset of contractions before intracellular P₀₂ began to fall. The TD was determined for each contractile period and added to the time at which the intracellular P₀₂ had fallen 50% from steady-state rest values to the new steady-state work value (obtained from the monoexponential curve fitting) to obtain the overall time required to achieve 50% response time (t₅₀).

Statistics. Repeated-measures analysis of variance was performed for the statistical analyses. In all analyses, the P < 0.05 level of significance was used. Results are reported as means ± SE.

RESULTS

Figure 1 illustrates the force produced during the experimental protocol for one typical single skeletal muscle fiber. The P₀ produced was not significantly different between the first stimulation period and the second in the 11 fibers used in the present study. In addition, the mean fall in force development that occurred within a contraction period (fatigue) was not significantly different between the two stimulation periods for the 11 fibers, with the ratio of force development at the end of the 3-min contractile period to the
Po for that contraction period being 83 ± 2 (SE) % in the first contractile period vs. 86 ± 3% in the second.

A representative example of the fall in intracellular Po2 at the onset of contractions for a single fiber is illustrated in Fig. 2. Intracellular Po2 was not significantly different between the first and second contractile periods at either the start of contractions (29 ± 2 vs. 35 ± 6 Torr) or the steady-state value attained during the contractile period (6 ± 1 vs. 7 ± 2 Torr). In all contraction periods, the fall in intracellular Po2 was well fit by a monoexponential function, with all fibers showing a TD in the fall of intracellular Po2 after the onset of contractions. Figure 3 demonstrates the difference in the TD between the first and second contractile periods, with the TD in the first period (13 ± 3 s) being significantly (P < 0.01) longer than in the second (5 ± 2 s). Finally, the time for the fall in intracellular Po2 to reach t50 after the onset of contractions, which includes the calculated TD, is illustrated in Fig. 4. This was significantly greater (P < 0.01) for the first compared with the second contraction period, being 28 ± 5 and 18 ± 4 s, respectively.

**DISCUSSION**

O2 uptake on-kinetics. The cause(s) of the delayed onset of oxidative phosphorylation at the onset of exercise or muscle contractions remains controversial (for review see Ref. 28). Because of the difficulty in determining intracellular oxygenation, it is uncertain whether O2 availability at the mitochondria is adequate during this transition period or whether other factors involved in the regulation of mitochondrial respiration are rate limiting. In previous studies utilizing an isolated in situ whole muscle model, it was demonstrated that, during transitions from rest to contractions of low metabolic intensity (~60% of peak VO2), increasing convective O2 delivery or improving peripheral O2 diffusion did not alter VO2 on-kinetics (10, 11). However, we recently demonstrated in the same muscle model (12) that an enhanced O2 delivery to contracting muscle at high stimulation intensities may indeed result in faster VO2 on-kinetics, confirming studies conducted in exercising humans (9, 16). Therefore, for transitions to exercise (or contractions) of low metabolic intensity, O2 availability to the mitochondria in whole muscle appears adequate at the onset of contractions, and other intracellular regulatory pathways represent the limiting factor(s) in determining VO2 on-kinetics. However, for higher metabolic transitions, O2 availability to the mitochondria together with the other intracellular constraints may determine VO2 on-kinetics.

Single fibers. Metabolic studies using human or whole muscle models can be difficult to interpret because of heterogeneity of blood flow and fiber recruitment patterns in the systems being examined. At the initiation of exercise, O2 availability at the microcirculatory level in vivo may be very heterogeneous, making the measurement of the actual O2 available to the mitochondria difficult to ascertain (6). To overcome these difficulties, the present study employed isolated
single skeletal muscle fibers so that specific conditions could be accurately controlled by adjusting the extracellular environment. Therefore, O_2 availability to the mitochondria was uniform around the fiber and determined solely by diffusive factors.

The amphibian muscle fibers used in the present study do not contain myoglobin; thus facilitated transport of O_2 within the cell was not present. With a uniform P_O2 around the cell and a constant diffusing capacity for O_2 within the cell, an increase in O_2 flux (V_O2) to the mitochondria can only be attained by reducing the intracellular P_O2. Although it has not been demonstrated in this preparation (or any other) whether the fall in intracellular P_O2 is directly correlated with a specific increase in V_O2, we have noted that intracellular P_O2 falls in a manner proportional to stimulation frequency (R. A. Howlett and M. C. Hogan, unpublished observations) in these single fibers, as would be expected if the fall in intracellular P_O2 were the primary means of an increased V_O2. In addition, the steady-state intracellular P_O2 achieved during the two contractile periods was not different, which would be expected for similar V_O2. Therefore, the intracellular P_O2 on-kinetics results found in the present study were likely directly correlated with V_O2 on-kinetics.

**Intracellular P_O2.** A number of methods have been used to estimate or measure intracellular P_O2 in skeletal muscle under various conditions (29), including O_2 microelectrodes (31), myoglobin saturation as determined by cryomicroscopy of frozen cell sections (8), and, more recently, spectroscopic relaxation determination of myoglobin saturation in whole muscle (20, 23). Each of these techniques has value under certain applications; however, none can provide a rapid, reliable measurement of intracellular P_O2 in single skeletal muscle cells over extended periods of time under conditions of rest and increased metabolic rate. Recently, a new method (using the O_2-dependent phosphorescence quenching of palladium-porphyrin compounds; Ref. 30) has been used successfully for measurements of microvascular P_O2 (24, 27, 34) and has recently been adapted for measuring intracellular P_O2 in single skeletal muscle fibers over extended periods of time (14).

Using this new method of measuring intracellular P_O2 (14), the data from the present study demonstrated that there was substantial O_2 available within the cell at the onset of contractions in these Xenopus single fibers. In fact, the P_O2 within the fiber did not begin to fall until after a significant TD after the initiation of contractions (see Figs. 2 and 3). It should be noted that this measured TD was not influenced by a slow response of the measuring system, because changes in intracellular P_O2 are immediate if the extracellular P_O2 is altered (14). After the initial TD, there was a monoexponential fall in intracellular P_O2 to a new steady-state value (see Fig. 2). The intracellular P_O2 t_{50} value obtained in the present study was likely indicative of the 50% time required to achieve a steady state of V_O2, because it has been demonstrated previously that V_O2 steady state was achieved in 1–1.5 min in these single skeletal muscle fibers (7, 21). The results from the present study make it clear that the time previously noted in these single isolated skeletal muscle fibers for oxidative respiration to reach a steady-state value (7, 21) was unrelated to O_2 limitation. In addition, the value of intracellular P_O2 t_{50} calculated in the present study during the first contractile period was very similar to V_O2 t_{50} values obtained in both human and whole muscle exercise (2, 3, 5, 9, 10, 11, 13, 16, 32, 33).

Therefore, one of the significant findings of the present study was to demonstrate that, because O_2 was not the rate-limiting step in the activation of oxidative phosphorylation at the onset of contractions in these Xenopus single muscle fibers, other factors related to the regulation of mitochondrial respiration were likely rate limiting and thereby determined the V_O2 response time. Whereas traditionally the changes in intracellular [ADP] (where brackets denote concentration) or the phosphorylation potential (ATP/ADP[P_i]) have been regarded as important regulators of cell respiration as metabolic demand increases, a number of investigators (2, 17, 18) have demonstrated a strong correlation between changes in phosphocreatine at the onset of exercise and the rate of adaptation of V_O2. In addition, it has been suggested that changes in cytosolic Ca^{2+} levels within the cell and particularly the mitochondria may regulate oxidative phosphorylation (1, 25). Adaptive changes in any of these putative regulators of mitochondrial respiration, or NADH availability, may require substantial time at the onset of contractions, thereby resulting in the V_O2 on-kinetic response. However, as we (12) and others (9, 16) have demonstrated, at very high metabolic rates, O_2 availability to the mitochondria may influence V_O2 on-kinetics, likely during the latter stages of mitochondrial activation when O_2 utilization becomes very high.

Finally, it should be noted that the O_2 surrounding the single fibers in the present study was uniform, unlike a single fiber in a whole muscle surrounded by a small number of capillaries. Under such in vivo conditions, the supply of O_2 to the mitochondria is likely less than the single-fiber preparation, so that it could be argued that O_2 supply to a working fiber in vivo may be more limiting than that seen in the preparation used in the present study. However, the similar onset t_{50} found in the present study compared with t_{50} values found in humans, whole muscle, and single fibers (2, 3, 5, 7, 9, 10, 11, 13, 16, 21, 32, 33) provides compelling evidence that even in vivo O_2 availability is adequate during the adjustment of V_O2 at the onset of exercise. In fact, with an abundant O_2 supply around an isolated single fiber, it would be expected that, if O_2 availability were truly rate limiting to mitochondrial respiration at the onset of contractions, a steady state of V_O2 would be achieved even more quickly than in vivo, which is not the case (7, 21).

**Prior activation of respiration.** A second important finding from the present study was that the second contractile period, which followed the first contractile period by 5 min, had a significantly faster rate of fall in intracellular P_O2 (see Figs. 3 and 4), again suggesting a
faster onset of $\dot{V}O_2$. This phenomenon of faster $\dot{V}O_2$ on-kinetics after a prior period of contractile activation has also been recently demonstrated in exercising humans (9, 16). As in the first contractile period, during the second contractile period there remained a TD after the initiation of contractions in which intracellular $P_O_2$ did not fall. Although this indicates that $O_2$ was abundant within the cell during the activation of mitochondrial respiration in the second contractile period, the smaller TD and calculated $t_{50}$ in the second period indicates that the increase in utilization of $O_2$ occurred at a faster rate as oxidative phosphorylation was likely activated more quickly.

It should be noted that the majority of the change in the $t_{50}$ between the first and second contraction periods was due to the significantly smaller TD during the second period. Of the ~10-s difference in the $t_{50}$ between the two contractile periods, the smaller TD in the second contractile period accounted for 8 of the 10 s. This indicates that the faster activation of mitochondrial respiration in the second contraction period was primarily a result of a more rapid initial activation and that, once activation occurred, the time to steady state was not very different in the two contractile periods. Because $O_2$ availability to the mitochondria was adequate during the onset of contractions in both periods, one of the putative regulators of oxidative phosphorylation previously discussed was likely activated to a greater degree in the second work bout than the first. However, one possibility in the present study was that pyruvate dehydrogenase (PDH) was in a more active state before the second contractile period, allowing a greater delivery of acetyl-CoA to the tricarboxylic acid cycle from pyruvate. This would result in a more rapid delivery of NADH to the electron transport chain and thereby an accelerated onset of oxidative phosphorylation. Although it has not been directly demonstrated that muscle with greater PDH activation at the onset of exercise has faster $\dot{V}O_2$ on-kinetics, it has been shown that, when PDH is activated before exercise or contractions, there is less subsequent perturbation of intracellular homeostasis (15, 22, 26). The results from these prior studies (15, 22, 26) suggest that oxidative respiration was likely initiated more rapidly as a result of the PDH being in a more active form, thereby decreasing the reliance on nonoxidative metabolism (i.e., phosphocreatine hydrolysis and anaerobic glycolysis) during the transition from the rest-to-work steady state.

Conclusion. The results of this study demonstrated that, in single Xenopus muscle fibers, $O_2$ availability to the mitochondria was adequate during the adjustment of mitochondrial respiration to a step increase in ATP demand and that a prior activation of the single fiber resulted in a more rapid fall in intracellular $P_O_2$ during a subsequent contractile period. These results suggest that other regulators of mitochondrial respiration, rather than $O_2$ availability, determine the rate at which oxidative phosphorylation is adjusted during the transition from rest to exercise and that prior activation of these regulators results in more rapid on-kinetics.

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REFERENCES


